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(54) ANTIVIRAL POLYMER

(71) We, HOECHST AKTIENGESELLSCHAFT, a body corporate organised according to the laws of the Federal Republic of Germany, of 6230 Frankfurt (Main) 80, Postfach 80 03 20, Federal Republic of Germany, do hereby declare the invention for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The inventon relates to polymers having anti-viral activity.

It has been proposed that copolymers of divinyl ether and maleic acid anhydride can be used for combating the virus provoking aphthous fever (of. U.S. Patent Specification No. 3,624,218). It has also been proposed that polyvinyl-sulphonic acid of various degrees of sulphonation and different molecular weights can be used for prophylactic steps against this virus (cf. U.S. Patent Specification No. 3,466,365). Moreover, it has been shown that polyacrylic acid has anti-viral activity (of. Journal of Virology, Vol. 2, no. 9 (1968), pages 878 and 886, Symp. Series immunobiol. Standard., Vol. 14 (1970), page 221). All the afore-mentioned polymers have the common feature that they are obtained by free radical polymerization and that due to this type of preparation they have a broad molecular weight distribution.

The present invention provides a polyacrylic acid which has an average moelcular weight, calculated from viscosity measurements, within the range of from 5,000 to 40,000, a polydispersity (as hereinafter defined) within the range of from 1.1 to 2, and wherein at least 75% of the carboxylate groups are in an isotactic configuration and not more than 5% of the carboxylate groups are esterified.

The invention also provides esters and salts of this polyacrylic acid.

The average molecular weight of the polyacrylic acid as calculated from viscosity measurements, is preferably from 8,000 to 30,000, and especially from 10,000 to 25,000, the polydispersity is preferably from 1.1 to 1.5 and the amount of carboxylate groups in isotactic configuration is preferably from 90 to 98 weight percent.

To determine the average molecular weight, the polymer is dissolved in chloroform and the viscosity of the solution is determined at polymer concentrations of 0.3 g/100 ml, 0.6 g/100 ml and 0.9 g/100 ml at a temperature of 30°C in each case. After having graphically determined the viscosity limit $[\eta]$ at which the concentration c=0, the average molecular weight $\overline{M}$ is calculated from the equation

$$[\eta]=1.4 \times 10^{-4} \times \overline{M}^{-0.72}$$

(cf. Macromolekular Syntheses, Vol. 1 (1963), page 25). The average molecular weight calculated by viscosity measurements is usually slightly less than the corresponding weight-average molecular weight. By "polydispersity" there is to be understood the quotient of the weight-average molecular weight ($\overline{M}_w$) and the number-average molecular weight ($\overline{M}_n$). $\overline{M}_n$ is determined by means of gel permeation chromatography. The proportion of the polymer in isotactic configuration is determined by means of nuclear magnetic resonance spectroscopy.

The invention also provides a process for preparing a polyacrylic acid according to the invention, which comprises polymerising an ester of acrylic acid with an alcohol branched in the α-position from a solution thereof in an inert anhydrous solvent with the exclusion of oxygen and in the presence of an anionically active catalyst at a temperature within the range of from 0° to −80°C, to obtain a polyacrylate having a viscosity average molecular weight corresponding to that defined above for the polyacrylic acid of the invention, removing any atactic polymer present in excess of 25% by weight, subjecting the resulting substantially isotactic polyacrylic acid to precipitating fractionation if the polydispersity is greater than 2, and subjecting the resulting polacrylic acid ester to acid hydrolysis until not

more than 5% of the ester groups remain.

The steps of removing excess atactic polymer and of ensuring the polydispersity is not more than 2 may be carried out in the reverse order, if desired.

The preferred monomers are esters of acrylic acid with monovalent alcohols branched in the α-position and having 3, 4, 5 or 6 carbon atoms, for example, cyclohexyl acrylate, isopropyl acrylate, and tert.-butyl acrylate. Alkali metal alcoholates having up to 4 carbon atoms, for example, sodium methylate, sodium ethylate, sodium isopropylate and sodium-tert.-butylate are suitable catalysts as they are anionically active; also suitable are aliphatic alkali metal alkyls, for example, tert.-butyl lithium and isopropyl sodium, and, preferably, Grignard compounds having up to 7 carbon atoms, for example, ethyl magnesium bromide, isopropyl magnesium bromide, tert.-butyl magnesium chloride, phenyl magnesium bromide, benzyl magnesium bromide and benzyl magnesium chloride. The amount of catalyst used is generally from 0.01 to 05, preferably from 0.05 to 0.3 mole per mole of monomer.

Polymerization of the acrylate is preferably carried out at a temperature within the range of from −60 to −80°C. The inert organic solvent used must have a melting point below −80°C, and is especially a monoalkylbenzene for example, toluene, ethylbenzene or propylbenzene. The polymerization generally requires from 10 minutes to 24 hours, preferably from 1 to 6 hours, depending on the quantity of monomer used.

More than 25% by weight of the resulting polyacrylic acid ester may be in the atactic form, and this proportion must be reduced to 25% or less, preferably from 10 to 2% by weight. The atactic polymer may be removed from the reaction mixture to a great extent, for example, by agitation with isopropanol at a temperature of from 15 to 30°C, preferably at room temperature, generally for a period from 12 to 24, preferably from 14 to 18 hours.

The weight average molecular weight of resulting polyacryate can be adjusted by means of the quantity of the catalyst used, the viscosity average molecular weight generally being from 7,900 to 64,000, preferably from 13,000 to 40,000.

The resulting essentially isotactic polyacrylate is subjected to acid hydrolysis until not more than 5% of the ester groups remain. Suitable media are especially mixtures of water and strong organic acids, the quantity of water being from 60 to 1%, preferably from 40 to 15%, by volume and the quantity of acid being from 40 to 99%, preferably from 60 to 85%, by volume. As the strong organic acid there is especially used an acetic acid having one, two or three halogen substituents for example, monochloroacetic acid, monofluoroacetic acid, trichloroacetic acid and trifluoroacetic acid. The quantity of the medium is preferably in an excess by weight of from 5 to 20, preferably from 8 to 15 calculated on the quantity by weight of the polymer to be hydrolysed, i.e. a solution of the polymer is used which contains from 5 to 20, preferably from 7 to 12 weight percent of polyacrylate. The hydrolysis is generally carried out at the boiling temperature of the medium, preferably at a temperature of from 80 to 120°C; the operation lasts normally from 50 to 90, preferably from 60 to 80 hours. Subsequently, the resulting polyacrylic acid may be purified by means of dialysis against water and submitted to vacuum freeze drying.

If the polydispersity of the polyacrylate formed as intermediate in the process of the invention exceeds 2, the product is submitted to precipitating fractionation. One of the above mentioned inert aromatic solvents may be used, as the solvent, ain aliphatic hydrocarbon having from 5 to 8 carbon atoms and preferably being straight chained, e.g. *n*-pentane, *n*-hexane, *n*-heptane and *n*-octane, being used as the precipitating agents.

The polyacrylic acid may be neutralized to give a salt by means of an organic or inorganic base, especially by means of a salt with basic action, preferably an alkali metal salt of a polybasic mineral acid, for example, sodium hydroxide, potassium hydroxide, or ammonia, or a primary or secondary aliphatic or aromatic amine having hydrocarbon radical(s) each having up to 6, preferably 1, 2 or 3 carbon atoms, e.g. methylamine, ethylamine, propylamine, aniline, dimethylamine, diethylamine and dipropylamine, furthermore sodium dihydrogen phosphate, disodium hydrogen phosphate, ammonium dihydrogen phosphate, sodium carbonate and sodium hydrogen carbonate.

The salt of polyacrylic acid, preferably an alkali metal salt thereof, may be used for prophylactic treatment to combat certain viral infections and also for therapeutic treatment immediately following infection by certain viruses.

The antiviral efficiency of an isotactic polyacrylic acid having a high degree of isotacticity and a narrow range of molecular weight distribution is greater at the same weight average molecular weights, than a corresponding isotactic polyacrylic acid having a broader range of molecular weight distribution. Moreover, it is much higher than that of known atactic polyacrylic acids of identical molecular weight, regardless of whether the atactic polyacids have a narrow or a broad range of molecular weight distribution.

The isotactic polyacrylic acid of the invention shows *in vivo* better antiviral properties than atactic material. From the results of experiments *in vitro* it has been proposed that either a direct physical bond is formed between

the polymer and the virus, the inactivation of the virus depending on the pH value or, due to an electrostatic modification of the cell surface, the penetration of the virus into the cell and thus the infection is impeded. The isotactic polyacrylic acid *in vivo* reduces the consequences of certain viral infections after they are manifested, either by inhibition of the proliferation of virus or by increased resistance of the organism due to stimulation of the reticuloendothelial system (RES) including interferon induction and increased phagocytosis. The activity of the polyacrylic acid of the invention may be observed for up to 8 to 10 weeks after one single administration. The interferon induction is especially well discernible at high doses.

The anion of the polyacrylic acid of the invention is antivirally active, but the acid itself is not physiologically tolerable. The invention therefore provides a pharmaceutical preparation which comprises a salt of a polyacrylic acid of the invention in admixture or conjunction with a pharmaceutically suitable carrier. The preparation is usually in the form of a solution or suspension, and is prepared by adding the salt to a carrier so that the salt dissociates to give the anion. The salt may be formed *in situ* for example, by adding the acid to a buffer containing sodium or potassium salts.

A suitable carrier is, for example, water or an aqueous buffer solution; a glycol, for example, propylene glycol; an alcohol, for example, glycerine; an ester, for example, diethyl carbonate; or an oil, for example, peanut oil or sesame oil. The pH value of the solution or the suspension is from 5 to 7.5—preferably from 6 to 7. The solution or suspension may be administered either intravenously (iv), intraperitoneally (ip), intramuscularly (im), subcutaneously (sc) or, in some cases, *per os*. The dose administered varies, but is generally from 5 to 1,500 mg/kg, preferably from 50 to 500 mg/kg; for intravenous administration a dose of from 80 to 150 mg/kg is especially recommended, for intraperitoneal application a dose of from 50 to 150 mg/kg, for intramuscular administration a dose from 150 to 500 mg/kg and for subcutaneous application a dose of also 150 to 500 mg/kg. The solution or suspension has a volume of from 0.05 to 1.0—preferably from 0.1 to 0.5 ml. In addition to the salt of an acid of the invention, there may be included in the preparation further therapeutic agents, for example, antibiotics, analgesics and hypnotics.

The anion of polyacrylic acid according to the invention is effective against a wide range of viruses. Positive reactions are observed against RNA viruses; for example, Columbia SK virus, Encephalomyocarditis virus (EMC —Theiler), influenza A virus/PR8 and vesicularstomatitis virus/strain Indiana, and against DNA viruses, for example, Vaccinia virus/P 71. The action against Columbia SK virus and EMC virus was tested on NMRI mice from SPF breeding stock having a weight of from 76 to 20 g, the test consisting of administering to the mice from 10 to 20 lethal doses/50% (LD_{50} according to Reed and Muench) of a cerebral suspension of the virus strain to be examined. A "cerebral suspension" is a suspension in a buffered sodium chloride solution of cerebra of mice which had been infected with the virus in question, and had fallen ill. Untreated mice were killed by the infection about 7 to 9 days after administration of the cerebral suspension, death being caused by mounting paralysis and break-down of respiration. Death by infection can be prevented with a great degree of certainty, if the test mice are treated with the anion of polyacrylic acid according to the invention within a period of from several weeks prior to 16 hours after infection.

The following Examples illustrate the invention:

EXAMPLE 1.

Polyacrylic acid having various molecular weights were prepared as follows:

Various amounts of a 2,3M-phenylmagnesium bromide solution in diethyl ether were added in each case to 500 ml of absolute toluene which had been freed from oxygen by introducing nitrogen. The mixture was cooled to −78°C by means of a cooling bath and after addition of 43 ml (0.34 mole) of isopropyl acrylate the reaction mixture was maintained at this temperature for 24 hours. By adding the resulting mixture to a tenfold quantity by volume of a mixture of methanol, water and hydrochloric acid(proportion by volume 20:4:1), the polymer formed precipitated. The yields are given in Table I. The precipitated polymer was then stirred for 12 hours in a tenfold quantity by volume of isopropanol at room temperature. The average molecular weight of the polyacrylate was determined by dissolving 0.3 g, 0.6 g and 0.9 g of the polymer in 100 ml each of chloroform and by measuring the viscosity of the solution at a temperature of 30°C; the boundary viscosity $[\eta]$ at the concentration of c=0 was determined graphically and the molecular weight $\bar{M}$ is determined by caculation (results see table 1). The polydispersity of the polymer was determined by means of gel permeation chromatography. As the polydispersity was greater than 2, an additional precipitating fractionation with benzene as solvent and *n*-hexane as precipitating agent was subsequently carried out.

For hydrolysis, 5 g each of the resulting essentially isotactic polyisopropyl acrylate were dissolved in a mixture of 80 ml of trifluoroacetic acid and 20 ml of water, and the resulting solution was heated under nitro-

gen reflux for 72 hours. The polyacrylic acid precipitating from the reaction mixture was separated from the mixture by filtration. It was dissolved in hot water, submitted to dialysis against water and to vacuum freeze drying. There was obtained 3.1 g (98.5% of the theoretical yield) of isotactic polyacrylic acid having a hydrolysis ratio of 95%.

For therapeutic or prophylactic application as the anion a quanity of from 0.3 to 30.0 mg of polyacrylic acid was dissolved in each case in one millilitre of an aqueous buffer solution containing in 100 ml 5.368 g of sodium dihydrogen phosphate and 8.746 g of disodium hydrogen phosphate and having a pH value of 7.0.

TABLE 1

Yield of polyacrylate

Example	initiator solution (ml)	molecular weight	polydispersity	isotactic portion (weight %)	yield (weight %)
a.*	5	75,000	1.9	>95	60
b.	10	30,000	1.3	>95	65
c.	13	20,000	1.2	>95	70
d.	20	14,000	1.3	>95	70
e.	30	8,000	1.4	>95	50
f.	40	7,000	1.4	>95	55

*Comparative Example.

EXAMPLE 2.

The following tests demonstrate the activity of the polyacrylic acid of the invention and that this activity depends on the dose administered. An essentially isotactic polyacrylic acid having an average molecular weight of 16,000, a polydispersity of 1,2, 95% of the side chains in the isotactic configuration and not more than 5% of esterified acid groups was prepared according to the method described in Example 1. A series of solutions containing from 0.3 to 10 mg/ml of this acid in an aqueous buffer as described in Example 1, was prepared. Constant volumes of these solutions with various concentration of the polyacrylic acid administered were administered by subcutaneous injection to 4 groups of 10 mice each. 24 hours after the injection the various groups of mice, as well as a control group of 10 untreated mice were infected with Columbia SK virus. The activity of the anion of polyacrylic acid and the dose dependence can be seen from the survival ratio shown in Table 2.

TABLE 2

Example	Dose (mg/mouse)	Survival ratio (%)
a.	—	0
b.	0.3	0
c.	1.0	20
d.	3.0	70
e.	10.0	80

EXAMPLE 3.

The following experiments demonstrate that the activity of the anion of polyacrylic acid depends on its polydispersity: Use is made of solutions as described in Example 2. Various amounts of this solution were administered to 3 groups of 10 mice each by subcutaneous injection. 3 Further groups of 10 mice each, were treated in the same manner, except that to the saline solution was added a polyacrylic acid having a molecular weight (MW) of 17,000 and a polydispersity of 10. 24 Hours after the injection the test mice were infected with 10 LD_{50} Columbia SK virus, as were a group of 10 untreated mice which served as a control (see a). The survival ratio (see Table 3) shows the activity of the anion of polyacrylic acid.

TABLE 3

Experiment	Polyacrylic acid		Dose (mg/mouse)	Survival ratio %
	Molecular weight	Polydispersity		
a	—	—	—	—
b	16,000	1.2	0.3	0
c	16,000	1.2	1.0	20
d	16,000	1.2	3.0	70
e	17,000	10	0.3	0
f	17,000	10	1.0	10
g	17,000	10	2.0	40

EXAMPLE 4.

The following experiment demonstrate that the activity of the anion polyacrylic acid depends on the molecular weight of the polyacrylic acid and on the tacticity thereof:

A saline solution identical to that defined in Example 1 was used, the average molecular weight values and the isotactic proportions of the polyacrylic acid varying however; the polydispersity was from 1.2 to 1.4 and the unsaponified portion is a maximum of 5 weight percent. Various quantities of the saline solution were administered to groups of 10 mice each by subcutaneous injection, and 24 hours after the injection the mice were infected with 10 LD_{50} of Columbia SK virus. 14 days later the harmonic average values of the survival period of the corresponding groups were compared.

The harmonic average M_h of n observed values x_1; x_2, ..., x_n is the quotient of the number n of observations and the sum of the reciprocal individual values

$$M_h = \frac{n}{\frac{1}{x_1} + \frac{1}{x_2} + \ldots + \frac{1}{x_n}}$$

It is especially useful to determine this average if time is a characteristic factor to be observed. The advantage of this method is the fact that "indefinite" periods can be included in the calculation, i.e. dying and surviving animals can be included in the results, because the reciprocal value of "indefinite"

$$\frac{1}{\infty} = 0$$

(cf. L. Cavalli-Sforza: Grundbegriffe der Biometrie, G. Fischer Verlag, Stuttgart 1964).

The accompanying charters 1 to 4 show that the harmonic average of the survival periods depends on the molecular weight and the tacticity of the polyacrylic acid salt.

Fig. 1)

curve a): Polyacrylic acid with a molecular weight of 51,500, at least 95% isotactic, polydispersity 1.4;

curve b): Polyacrylic acid with a molecular weight of 56,000, a maximum of 5% isotactic, polydispersity 1.2.

Fig. 2)

curve a): Polyacrylic acid with a molecular weight of 24,600, at least 95% isotactic, polydispersity 1.3;

curve b): Polyacrylic acid with a MW of 25,600, a maximum of 5% isotactic, polydispersity 1.4.

Fig. 3)

curve a): Polyacrylic acid with a molecular weight of 11,700, at least 95% isotactic, polydispersity 1.2;

curve b): Polyacrylic acid with a molecular weight of 14,000, a maximum of 5% isotactic, polydispersity 1.3;

curve b): Polyacrylic acid with a molecular weight of 15,900, a minimum of of 95% isotactic, polydispersity 1.2;

curve d: Polyacrylic acid with a molecular weight of 15,700, a maximum of 5% isotactic, polydispersity 1.3.

Fig. 4)

curve a): Polyacrylic acid with a molecular weight of 7,500, a minimum of 95% isotactic, polydispersity 1.3;

curve b): Polyacrylic acid with a molecular weight of 8,600, a maximum of 5% isotactic, polydispersity 1.2.

WHAT WE CLAIM IS:—

1. A polyacrylic acid which has an average molecular weight, calculated from viscosity measurements, within the range of from 5,000 to 40,000, a polydispersity (as hereinbefore defined) within the range of from 1.1 to 2, and wherein at least 75% of the carboxylate groups are in an isotactic configuration and not more than 5% of the carboxylate groups are esterified.

2. A polyacrylic acid as claimed in claim 1, wherein the average molecular weight is within the range of from 8,000 to 30,000.

3. A polyacrylic acid as claimed in claim 2, wherein the average molecular weight is within the range of from 10,000 to 25,000.

4. A polyacrylic acid as claimed in any one of claims 1 to 3, wherein the polydispersity is within the range of from 1.1 to 1.5.

5. A polyacrylic acid as claimed in any one of claims 1 to 4, wherein from 90 to 98% of the carboxylate groups are in isotactic configuration.

6. A salt of a polyacrylic acid as claimed in any one of claims 1 to 5.

7. An alkali metal salt of a polyacrylic acid as claimed in any one of claims 1 to 5.

8. An ester of a polyacrylic acid as claimed in any one of claims 1 to 5.

9. A process for preparing a polyacrylic acid as claimed in claim 1, which comprises polymerising an ester of acrylic acid with an alcohol branched in the α-position from a solution thereof in an inert anhydrous solvent with the exclusion of oxygen and in the presence of an anionically active catalyst at a temperature within the range of from 0° to −80°C, to obtain a polyacrylate having an average molecular weight, calculated by viscosity measurements, corresponding to the average molecular weight for polyacrylic acid as defined in claim 1, removing any atactic polymer present in excess of 25% by weight, and subjecting the resulting substantially isotactic polyacrylic acid ester to precipitating fractionation if the polydispersity is more than 2, and subjecting the resulting polyacrylic acid ester to acid hydrolysis until not more than 5% of the ester groups remain.

10. A process as claimed in claim 9, wherein the acrylic acid ester has 3 to 6 carbon atoms in the ester moiety.

11. A process as claimed in claim 10, wherein the acrylic acid ester is isopropyl acrylate.

12. A process as claimed in any one of claims 9 to 11, wherein the anionically active catalyst is an alkali metal alcoholate, an alkali metal alkyl, or a Grignard compound having up to 7 carbon atoms.

13. A process as claimed in any one of claims 9 to 12, wherein from 0.01 to 0.5 mole of catalyst is used per mole of acrylic acid ester.

14. A process as claimed in claim 13, wherein from 0.5 to 0.3 mole of catalyst is used per mole of acrylic acid ester.

15. A process as claimed in any one of claims 9 to 14, wherein the atactic polymer is removed by fractionation.

16. A process as claimed in claim 15, wherein the atactic polymer is removed by shaking with isopropanol at a temperature within the range of from 15° to 30°C.

17. A process as claimed in claim 9, conducted substantially as described in Example 1 herein.

18. A polyacrylic acid as claimed in claim 1, whenever produced by a process as claimed in any one of claims 9 to 17.

19. A pharmaceutical preparation which comprises a salt of a polyacrylic acid as claimed in any one of claims 1 to 5 or claim 18 in admixture or conjunction with a pharmaceutically suitable carrier.

20. A pharmaceutical preparation as claimed in claim 19, in the form of a solution or suspension.

21. A pharmaceutical preparation as claimed in claim 19 or claim 20, which also comprises one or more further therapeutically active compounds.

22. A pharmaceutical preparation as claimed in claim 21, wherein the other compound(s)

is or are selected from antibiotics, analgesics and hypnotics.

23. A pharmaceutical preparation as claimed in one one of claims 19, to 22, in unit dosage form.

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1469845 COMPLETE SPECIFICATION

4 SHEETS *This drawing is a reproduction of the Original on a reduced scale*

Sheet 1

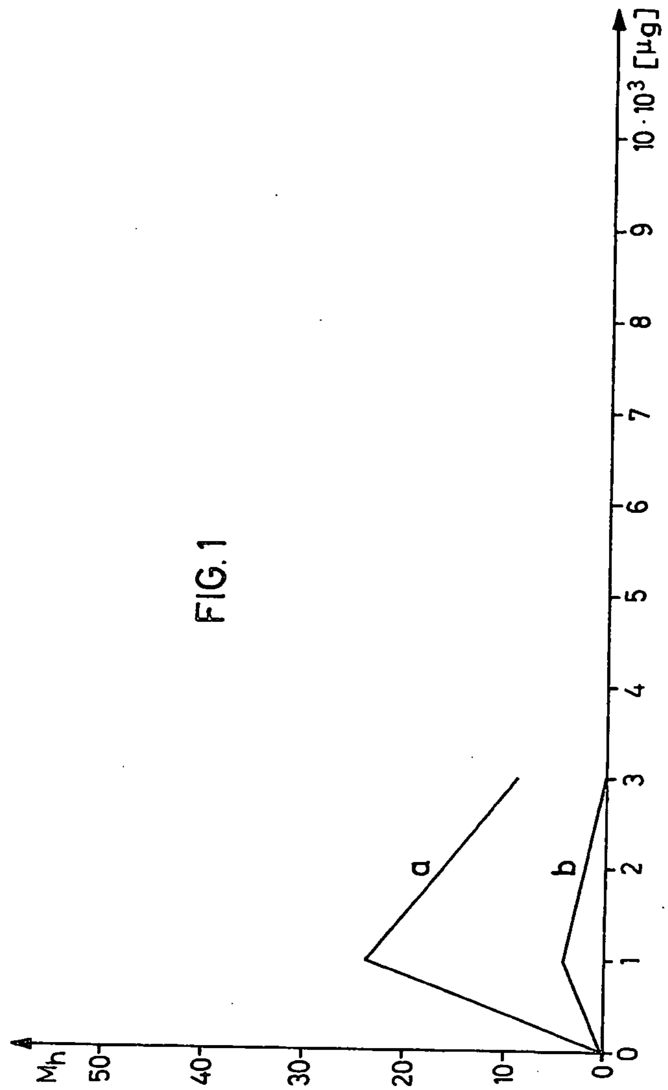

FIG. 1

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Sheet 2

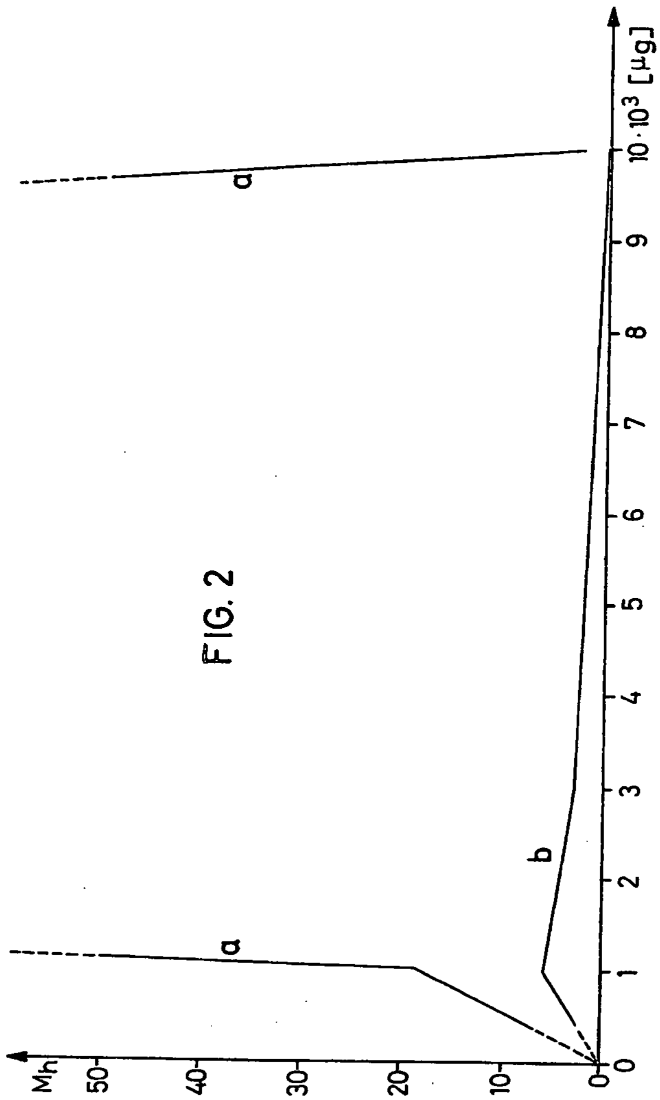

FIG. 2

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Sheet 3

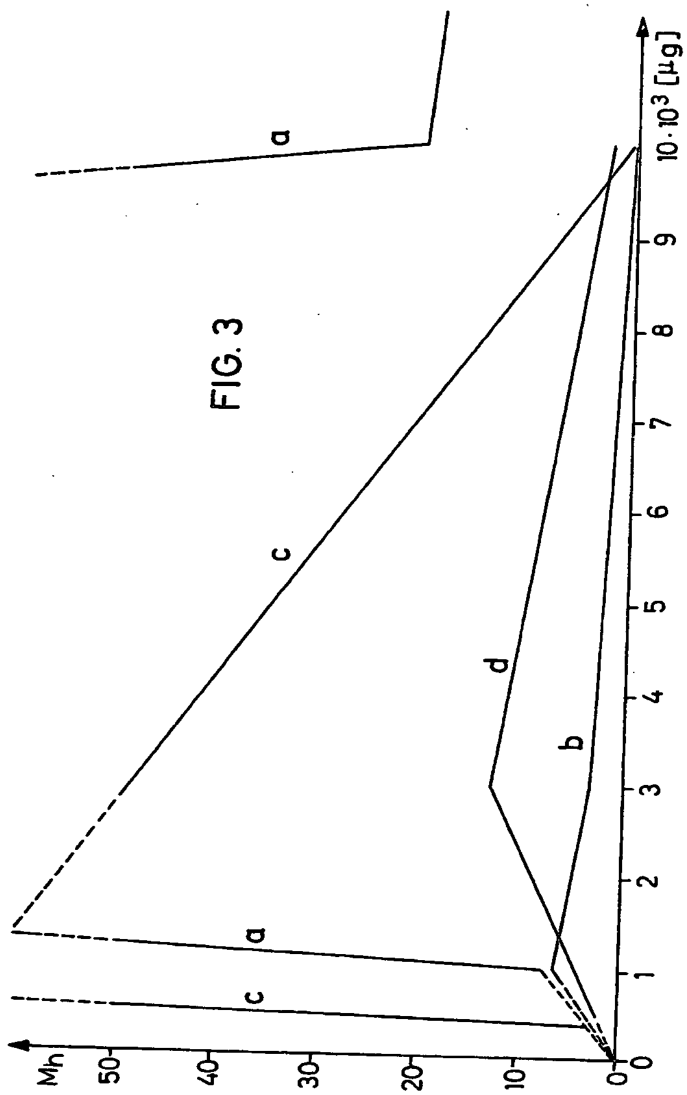

FIG. 3

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Sheet 4

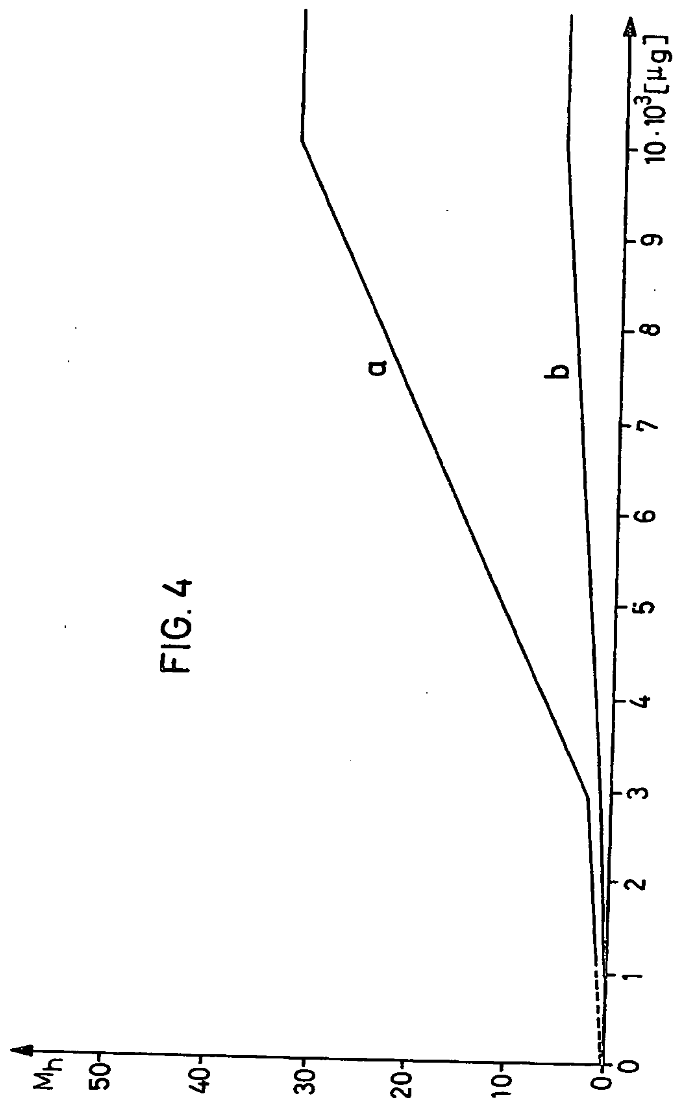

FIG. 4